Conduction and Block by Organic Cations in a K$^+$-selective Channel from Sarcoplasmic Reticulum Incorporated into Planar Phospholipid Bilayers

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ABSTRACT   A collection of organic cations has been used to probe the gross structural features of the ionic diffusion pathway in a K$^+$-selective channel from sarcoplasmic reticulum (SR). Channels were incorporated into planar phospholipid bilayer membranes, and single-channel currents were measured in the presence of ammonium-derived cations in the aqueous phases. Small monovalent organic cations are able to permeate the channel: the channel conductance drops sharply for cations having molecular cross sections larger than 18–20 Å^2. Impermeant or poorly permeant cations such as tetraethylammonium, choline, and glucosamine, among others, block K$^+$ conduction through the channel. This block is voltage dependent and can be described by a one-site, one-ion blocking scheme. 19 monovalent organic cations block primarily from the *trans* side of the membrane (the side defined as zero voltage), and much more weakly, if at all, from the *cis* side (to which SR vesicles are added). These blockers all appear to interact with a site located at 63% (average value) of the electric potential drop measured from the *trans* side. Furthermore, block by 1,3-*bis*[tris(hydroxymethyl)-methylamino] propane (BTP) shows that the presence of a blocking ion increases the duration of the apparent open state, as expected for a scheme in which the blocking site can be reached only when the channel is open. The results lead to a picture of the channel containing a wide (at least 50 Å^2) nonselective *trans* entry in series with a narrow (20 Å^2) constriction.

INTRODUCTION

During the past few years there has been a growing interest in the use of ammonium-derived organic cations as probes for the atomic dimensions, selectivity, and molecular structure of ionic channels (Hille, 1975; Armstrong, 1975a and b; Moreno and Diamond, 1975; Rojas and Rudy, 1976; Watanabe and Narahashi, 1979; Dwyer et al., 1980; Kirsch et al., 1980; Farley et al.,

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1981; French and Shoukimas, 1981; Swenson, 1981). Hille (1971a and b; 1973; 1975) measured the permeabilities of a series of monovalent organic cations in frog myelinated nerve, concluding that Na^+ and K^+ channels possess narrow selectivity barriers through which only a few species can permeate. The postulated dimensions (4 × 5 Å and 3 × 3 Å for the Na^+ and K^+ channels, respectively) are such that the alkali metal cations must cross these barriers in a partially dehydrated form. In the squid axon K^+ channel, block by tetraethylammonium (TEA) and its monoalkyl derivatives suggests that at least two regions in the channel can be distinguished: a wide inward-facing "mouth" of 8–9 Å diameter, and a narrower section controlling ionic selectivity (Bezanilla and Armstrong, 1972; Armstrong, 1975b). TEA, TEA derivatives, and impermeant alkali cations (Adelman and French, 1978) can enter the mouth of the K^+ channel, but are prevented from traversing the more selective region. Recently, work of Swenson (1981) and the work of French and Shoukimas (1981) indicates that the mouth of this channel could be as large as 10 × 12 Å.

This report describes a similar approach that we have taken with a K^+-selective channel from mammalian skeletal muscle sarcoplasmic reticulum (SR), a channel whose voltage-dependent gating mechanism and ionic conduction process have previously been described (Labarca et al., 1980; Coronado et al., 1980). The channel, which is studied by inserting fragmented SR into planar phospholipid bilayers by membrane fusion, allows conduction by monovalent cations but shows no measurable permeability to anions or divalent cations. Of the common monovalent cations, K^+ shows the highest conductance, 140 pS in 100 mM K^+. The conduction properties suggest that at most a single conducting ion can occupy the channel at a time.

The finding that Cs^+ ions block this channel (Coronado and Miller, 1979; Coronado et al., 1980) led us to search for other blockers. In this paper we study conduction and block by organic cations in order to estimate the internal dimensions of the SR channel. We show that the smallest cross section of the pore is ~20 $Å^2$, and that this constriction is probably located at 60–65% of the electric potential drop measured from the *trans* side of the membrane, i.e., the side opposite to the addition of SR vesicles. The channel appears to open to the *trans* aqueous solution via a cavity into which a large number of impermeant cations can enter and thereby block K^+ conduction. This *trans*-facing cavity is at least 7 Å in diameter.

MATERIALS AND METHODS

Chemicals

Chemicals used were reagent or purissimum grade and were obtained from the following sources. Aldrich Chemical Co., Milwaukee, Wis.: 4-aminopyridine and N,N-dimethylhydrazine HCl. Boehringer Mannheim Biochemicals, Indianapolis, Ind.: N,N-bis (2-hydroxyethyl)-glycine ("bicine"). Eastman Organic Chemicals, Rochester, N. Y.: aminoguanidine sulfate, dimethylammonium chloride, guanidine sulfate, tetrapropylammonium hydroxide, tetrabutylammonium hydroxide, and tetrapentylammonium bromide. Fisher Chemical Co., Boston, Mass.: diethylamine, hydrazine,

methylamine, and triethylamine. Fluka (Tridom Chemical Co., Hauppauge, N. Y.): 2-dimethylaminoethanol, diethanolamine, dimethylamine, ethanolamine, ethylamine, formamidine HCl, hydroxylamine sulfate, methylhydrazine, methylhydroxylamine HCl, tetraethylammonium hydroxide, and triethanolamine. Schwarz/Mann Div. Becton, Dickinson & Co., Orangeburg, N. Y.: Tris(hydroxymethyl)-aminomethane (Tris). Sigma Chemical Co., St. Louis, Mo.: arginine chloride, 1,3-*bis*(hydroxymethyl)methylamino propane, carbamylcholine, glucosamine, glucuronic acid, *N*-2-hydroxyethyl-piperazine-*N*'-2-ethanesulfonic acid (Hepes), and procaine.

Bilayers

Lipid bilayers were cast from *n*-decane solutions of phospholipid onto a 0.5-mm Diam aperture in a polystyrene cup, as described (Labarca et al., 1980). The composition of the planar bilayers was 95% phosphatidylethanolamine (PE)/5% phosphatidylcholine (PC) purified from egg yolk. Neutral bilayers were used in order to minimize changes in surface potential because some experiments required variation in ionic strength. Preparation of SR vesicles from rabbit muscle and further details of the bilayer setup have been reported elsewhere (Miller and Rosenberg, 1979; Labarca et al., 1980).

Measurement of current through single channels was accomplished by allowing the fusion of only a single SR vesicle with the bilayer in the presence of an appropriate solution containing 0.3 mM Ca^{++} in the *cis* chamber. SR vesicles (1–5 μg/ml) were then added to this chamber, and conductance was monitored at +25 mV (*trans* chamber defined as zero voltage). After the first fusion event occurred, 0.5 mM EDTA was added to the *cis* chamber to prevent further fusion. When using organic cations as the only conducting ions, solutions were formed from sulfate or glucuronate salts of the desired organic cation, and buffered with 5 mM Hepes-Tris, pH 7.0. All solutions also contained 0.1 mM EDTA-Tris. Organic cations purchased in the halide form were converted to the sulfate or glucuronate salt using Dowex-1 anion exchanger columns. For blocking experiments, bilayers were formed in symmetrical solutions of 100 mM K^+ (sulfate or glucuronate salt), with 5 mM Hepes-KOH, pH 7.0, 0.1 mM EDTA-Tris, and the specified concentration of blocker. Solutions containing Cl^- were avoided because of the additional Cl^- conductance (not mediated by the K^+ channel) induced by SR vesicles (Miller, 1978). All experiments were carried out at room temperature, 21–23°C.

Molecular areas of cations were calculated from silhouette drawings of space-filling models (Koltun, 1965). The cross section in each case corresponds to the silhouette along the axis of the molecule containing the chemical group of largest area. In cases of asymmetric molecules containing a quaternary N, the molecular area of the N head was taken as the cross section. For symmetrical molecules, the cross section of the widest portion of the molecule was considered.

RESULTS

Single-Channel Conductance for Organic Cations

Fig. 1 shows recordings of single-channel currents resulting from incorporation of SR K^+ channels into bilayers, in the presence of 400 mM salts of various organic cations as the only cations present in solution. The vesicle incorporation process was similar to that seen in the presence of alkali metal cations or ammonium (Labarca et al., 1980; Coronado et al., 1980), and the voltage-dependent gating in the presence of organic cations was similar to that seen with K^+ as the conducting ion (data not shown). As with the alkali metal

cations and ammonium, the channel currents fluctuate randomly between two well-defined states. Among the organic cations tested, the largest conductance was seen for ammonium (156 pS), and substitution of a single H by NH_2 (hydrazinum) or CH_3 (methylammonium) produced substantial reductions in channel conductance (80 and 58 pS, respectively). Larger cations generated by substitutions of two H on the central nitrogen (dimethyl, diethyl,
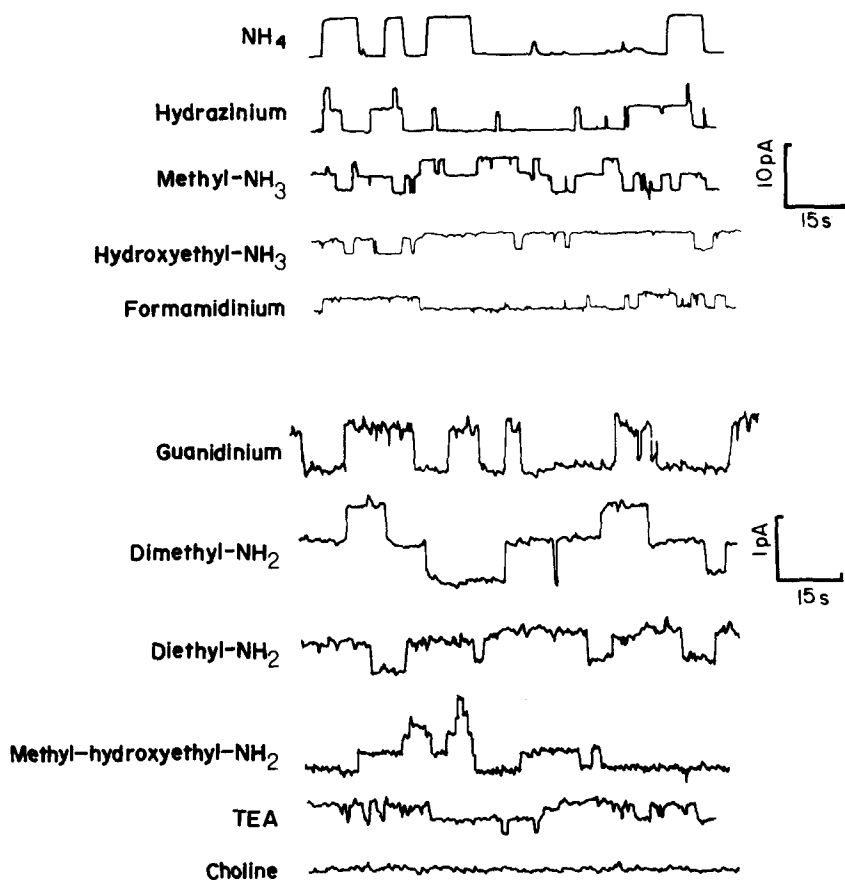

FIGURE 1.   Single-channel currents of organic cations. SR vesicles were fused into PE/PC bilayers in solutions of 0.4 M salts of organic cations indicated, as described in the text. In all cases, holding potential was −50 mV. The multiple current levels observed in some of the traces arise because of the presence of several channels in these bilayers.

methylhydroxyethyl) further decrease the channel conductance to the range of 10–20 pS. Finally, in the case of TEA, a bulky cation with a molecular diameter of ~6 Å, single channels can barely be discerned (<4 pS). No channel fluctuations at all can be seen in the presence of choline, or other large cations such as Tris and glucosamine (data not shown). In Table I, the

values of channel conductances are given for all conducting organic cations we have tested.

In Fig. 2, these conductances are plotted against the molecular cross sections of the cations. With the exception of a few ions, the channel conductance drops sharply for molecules with cross sections of 18 Å² or larger. This group of poorly conducting cations includes planar molecules such as guanidine

TABLE I

SINGLE-CHANNEL CONDUCTANCE IN 0.4 M ORGANIC
CATIONS

Cation	γ, pS	Code number (Fig. 2)
Ammonium	156±3	—
Methylammonium	58±2	1
Trimethylammonium	2–4	3
Choline	<1	4
Diethylammonium	9.0±0.8	6
Tetramethylammonium	1–3	TMA
Tetraethylammonium	2–4	TEA
Hydrazinium	81±1	—
Methylhydrazinium	40±2	10
N,N-dimethylhydrazinium	12±4	11
Hydroxyethylhydrazinium	10±1	12
Guanidinium	12.0±0.5	13
Methylguanidinium	3.8±0.4	14
Aminoguanidinium	2–5	15
Hydroxyguanidinium	4–5	16
Hydroxylammonium	9.2±0.3	17
Methylhydroxylammonium	5±2	18
Formamidinium	36±2	19
Ethanolammonium	50±2	20
Triethanolammonium	6±1	22
2-Methylaminoethanol	6.0±0.2	23
Tris-hydroxymethylaminomethane	<1	Tris
N,N-dimethylaminoethanol	<3	24

Channel conductances were measured as in Fig. 1. Each value, with standard error, represents 20–30 measurements in 2–5 different bilayers. In cases reporting a range of values (e.g., TEA), channel fluctuations were clearly present, but the baseline noise was too great to measure the conductance accurately. Cases labeled "<1 pmho" correspond to experiments in which there was no discernible noise (below 5 Hz) on the chart record; in these cases, we would have been able to detect a channel with 1 pmho conductance. Under the conditions here, all of the compounds with dissociable protons are at least 98% in the ionized state, except for hydroxylammonium, which is only 5% ionized.

derivatives, symmetrical quaternary amines, and large primary, secondary, and tertiary amines. Thus it is likely that these molecules are excluded solely on the basis of size, rather than by specific interactions with the channel. Assuming, then, that the nonhydrated size of these molecules is the important parameter, we postulate that the narrowest constriction of the SR K^+ channel is ~20 Å² in area.

would be a time-averaged value of the conductances of the unblocked and blocked states. This value will be voltage dependent, since the blocking site lies within the electric potential gradient inside the channel. These ideas are easily collected into a simple equilibrium treatment of voltage-dependent block (Woodhull, 1973; Coronado and Miller, 1979; White and Miller, 1981), leading to a predicted voltage-dependence of the time-averaged channel conductance, $\langle \gamma \rangle$:
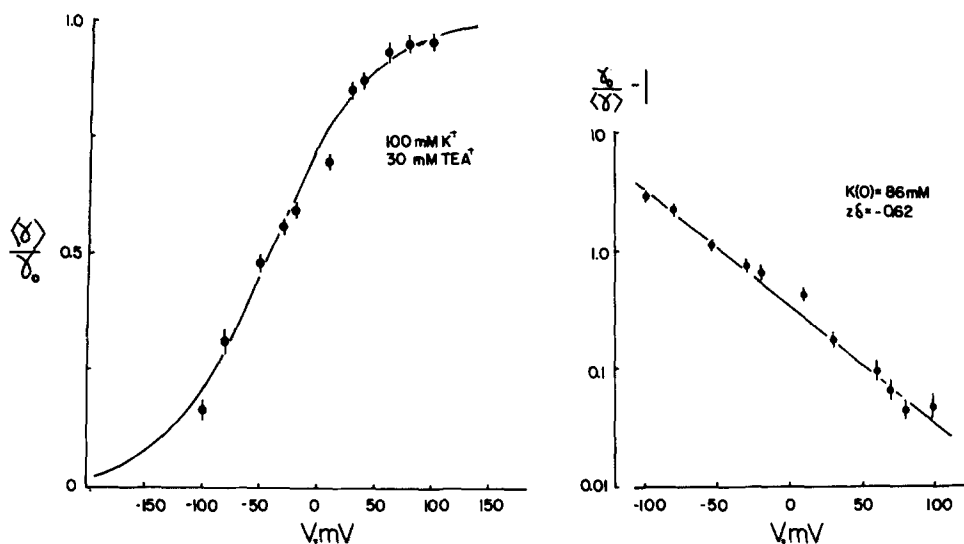

FIGURE 3.   Voltage-dependent block by TEA. Single-channel K^+ conductance was measured in PE bilayers, using symmetrical solutions containing 100 mM K^+ plus 30 mM TEA (sulfate salts). The left panel shows the ratio of apparent channel conductance, $\langle \gamma \rangle$, to the unblocked conductance, γ_o, measured in the range ± 100 mV. Each point represents the mean $\pm$ SEM of three to eight conductance determinations; points in these experiments were taken from three different membranes. The right panel shows same data plotted according to Eq. 2; this line determined blocking parameters $z\delta = 0.62$ and $K(0) = 86$ mM. These parameters are used to draw solid curve in the left panel according to Eq. 1. Unblocked channel conductance was 135 pS (measured at +100 mV).

where $[B]$, z, and $K(0)$ are the blocker concentration, valence, and zero-voltage dissociation constants, respectively, γ_o is the unblocked channel conductance, and δ is the fraction of the voltage drop experienced at the blocking site, as measured from the side of zero voltage, the *trans* side. This equation is conveniently linearized:

$$\ln[\gamma_o/\langle \gamma \rangle -1] = \ln([B]/K(0)) - z\delta \, FV/RT. \tag{2}$$

Thus, from an experiment such as that shown in Fig. 3, we can determine the blocker's dissociation constant at zero voltage, $K(0)$, and the "effective valence" of the blocking reaction, $z\delta$.

For the experiment of Fig. 3, we find that for TEA, $K(0)$ is 86 mM, and $z\delta$ is 0.62. Thus, in blocking SR channel, TEA is about 20-fold weaker than in the axon K^+ channel; the voltage dependence of the block suggests that the ion acts at a site ~60–65% down the voltage drop from the *trans* side. We have also found from control experiments that TEA and other organic blockers exert their effects mainly from the *trans* side (data not shown). Block can be

TABLE II

BLOCKING PARAMETERS FOR MONOVALENT ORGANIC
CATIONS

Cation	$z\delta$	K (0)
		mM
4-Aminopyridine	0.65±0.01	58±3
Bicine	0.64±0.02	500±20
Choline	0.61±0.03	230±15
Carbamylcholine	0.62±0.02	75±5
Glucosamine	0.59±0.02	117±5
Methylguanidine	0.65±0.04	120±5
2-Aminomethyl-1,3-propanediol	0.65±0.01	32±2
Tris	0.60±0.02	125±2
Triethanolammonium	0.65±0.04	87±5
Tetramethylammonium	0.68±0.01	500±10
Tetraethylammonium	0.62±0.01	85±2
Tetrapropylammonium	0.66±0.01	45±2
Tetrabutylammonium	0.65±0.01	26±3
Tetrapentylammonium	0.55±0.01	15±1
Hexyltrimethylammonium	0.66±0.05	53±5
Nonyltrimethylammonium	0.63±0.02	2±1
Decyltrimethylammonium	0.61±0.02	3.0±0.2
Arginine	0.44±0.02	108±8
Procaine	0.33±0.02	26±2

Blocking parameters were measured in PE membranes according to legend of Fig. 3. Errors correspond to the range of values consistent with rocking the line (according to Eq. 2) by eye about the least-squares regression line. Blockers were added to both sides of the membrane, at concentrations on the order of 20% of the $K(0)$. Channel conductance was determined in the range ± 100 mV. Each entry represents data taken at 10–12 voltages (4–15 determinations at each voltage), in at least three separate bilayers.

observed when the cations are added to the *cis* side, but our general experience has been that *cis* blocking is 10–50 times weaker than *trans* blocking. In all experiments reported here, the blocker is present symmetrically on both sides of the membrane, but at concentrations such that the *cis* blocking reaction contributes <2% of the blocking effect. Symmetric addition of blockers is always used so that problems due to asymmetric surface potentials due to binding of the blockers to the bilayer will not arise (Donovan and Latorre, 1979).

The blocking parameters $K(0)$ and $z\delta$ were measured for 19 different blockers (Table II), according to the methods shown above for TEA. In all cases, the unblocked K^+ conductance was in the range 130–140 pS, and the log plot (Eq. 2) was linear within the voltage range studied. With few exceptions, blocking organic cations appear to block at a site 60–65% down the electric potential gradient from the *trans* side, i.e., the effective valence of the block, $z\delta$, is 0.60–0.65, regardless of the size, shape, or hydrophobicity of the molecule. In several cases (procaine, arginine, tetrapentylammonium), the effective valence was significantly less than 0.62, but in no case have we seen an effective valence greater than 0.7. These results imply the existence of a
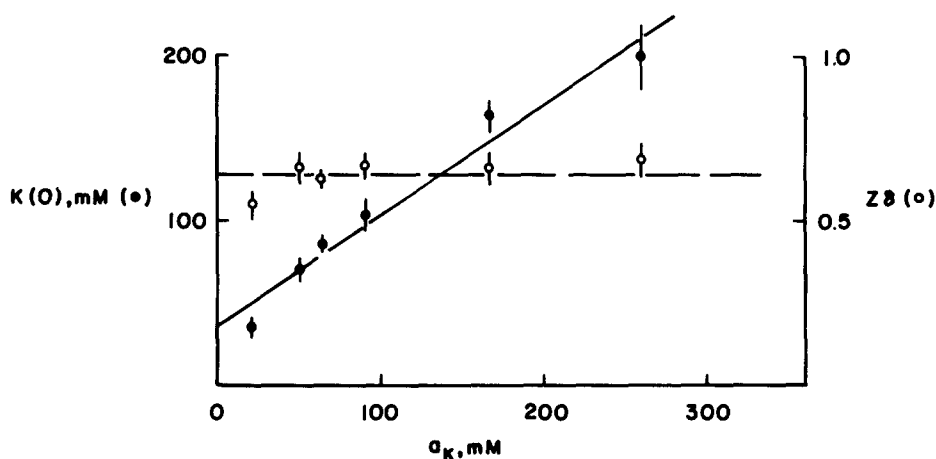

FIGURE 4. TEA block is competitive with K^+. Measurements of blocking parameters $z\delta$ and $K(0)$ were done in PE membranes as described in Fig. 3, except that various concentrations of K^+ were used, in the range 30–600 mM. Errors on the blocking parameters correspond to the range of values obtained by rocking the line according to Eq. 2, by eye, around the least-squares regression line. The least-squares regression line through the $K(0)$ data corresponds to dissociation constants for TEA and K^+ of 36 and 53 mM, respectively. The dissociation constant for K^+ was also determined from plots of unblocked γ_o vs. a_k, and was found to be 60 mM. K^+ activity coefficients in $SO_4^=$ solutions were taken from Tamamushi and Goto (1970).

wide, *trans*-facing cavity in which there is sufficient room to accommodate the blocker ion. They further imply that a well-defined blocking site exists at ~62% of the way down the voltage drop from the *trans* side. The fact that even the hydrophilic glucosamine ion can traverse >50% of the voltage drop argues that the *trans*-facing "mouth" of the channel is at least 6–7 Å wide.

If these blockers operate by entering the ionic diffusion region of the channel and thus excluding K^+ ions, then the block should be competitive with K^+. That this appears to be the case for TEA is demonstrated in Fig. 4. Here we examined TEA blocking at varying K^+ concentrations. We find that while $z\delta$ is independent of K^+ concentration, the apparent zero-voltage dissociation constant, $K(0)$, varies linearly with K^+ concentration, as expected for a

competitive scheme. The "true" dissociation constant for TEA is extrapolated to zero K^+ to be ~35 mM, whereas the dissociation constant for K^+ is calculated to be 53 mM, in excellent agreement with the dissociation constant for K^+ (50–60 mM) measured directly from the variation of K^+ conductance with K^+ activity (Coronado et al., 1980). We conclude that TEA block is competitive with K^+, as is also the case for Cs^+ block (Coronado et al., 1980) and for decamethonium block (Miller, 1982).

Coupling of Blocking and Gating: Block by BTP

The results above have been concerned only with the effects of blockers on the conductance of the open channel. But depending on their mechanism of action, blockers may also show effects on the gating of a channel (Ruff, 1977; Adams, 1977; Neher and Steinbach, 1978). If a blocker operates by diffusing into the normal K^+ diffusion pathway of the channel, then we can imagine the channel undergoing transitions among three states,

$$\text{CLOSED} \underset{\mu}{\overset{\lambda}{\rightleftharpoons}} \text{OPEN} \underset{\beta}{\overset{\alpha[B]}{\rightleftharpoons}} \text{BLOCKED}, \tag{3}$$
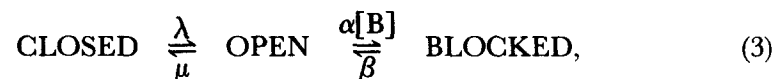
with only the open state having a measurable conductance. The rate constants, λ, μ, and β are first order, whereas α is second order. As mentioned above, if the OPEN $\rightleftharpoons$ BLOCKED equilibrium is fast compared with the time constant of the amplifier, then the observed "open state" of the channel represents a time average of the fully open and fully blocked states. Thus, with the blocker present, a channel spends part of its time in the blocked state, and so it will have less of an opportunity to close than will a channel without a blocker. According to scheme 3, then, a blocker will not only reduce the conductance of the "apparent open state", Eq. 1, but it will also increase the mean duration of this state. Scheme 3 predicts that the mean open time $\bar{\tau}_o$ increases linearly as a function of blocker concentration $[B]$:

$$\tau_o = \frac{1}{\mu} (1 + [B]/K(V)), \tag{4}$$

where $K(V)$ is the voltage-dependent blocker dissociation constant. Since a corresponding reduction in channel conductance under the same conditions follows from Eq. 1, we find that as the blocker concentration varies, the product of the open time and the conductance must remain constant:

$$\bar{\tau}_o \langle \gamma \rangle = \gamma_o/\mu. \tag{5}$$

Finally, scheme 3 explicitly demands that the mean closed time of the channel remain constant with blocker concentration.

In this section, we test these predictions for the blocker we have studied in most detail: 1,3-*bis*[Tris(hydroxymethyl)-methylamino]propane (*bis*-Tris-propane, or BTP). Recordings of channel fluctuations in the presence of BTP are shown in Fig 5. Increasing BTP concentration has two clear effects: a reduction in the open-state conductance, and a lengthening of the open time. The effects

are fully reversible, as shown by removal of BTP by perfusion of the *trans* chamber with blocker-free solution.

The three predictions above are satisfied. In Fig. 6 we see that the distributions of open and closed dwell times follow single exponential statistics, as shown before (Labarca et al., 1980). The addition of 4.5 mM BTP approximately doubles the mean open time, but leaves the mean closed time unchanged. Furthermore, we see (Fig. 7) that increasing BTP decreases the time-averaged channel conductance according to Eq. 1 and linearly increases the mean open time as demanded by Eq. 4; the value of $K(V)$ calculated from this straight line via Eq. 4 agrees precisely with the $K(V)$ calculated from the inhibition curve (3.3 and 3.5 mM, respectively). As predicted, the product
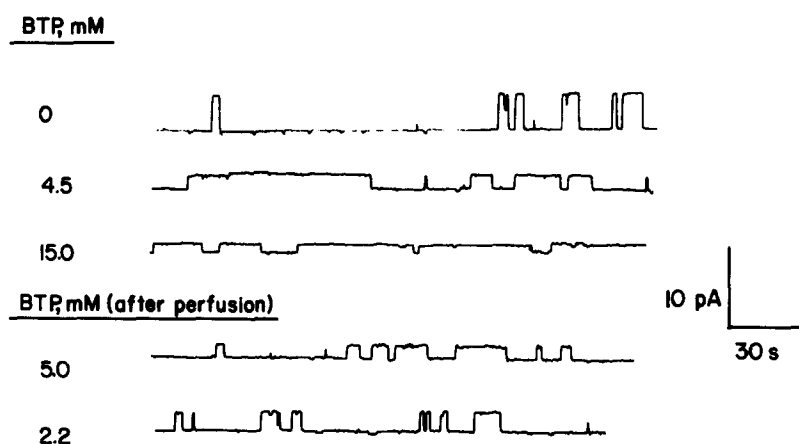

FIGURE 5.   Block of channels by BTP. A PE/PC membrane containing only one channel was examined in a 100-mM K^+ solution with various amounts of BTP added to both *cis* and *trans* sides of the membrane. A control trace was taken, and BTP was then added to the final concentration indicated. After 15 mM BTP had been added, both sides of the membrane were perfused with known volumes of solution containing BTP to give the final concentrations indicated. (Perfusion of chambers and previously been calibrated with dyes.) Holding voltage was −30 mV.

$\bar{\tau}\langle\gamma\rangle$ remains constant, with a value of 635 pF. We therefore conclude that scheme 3 is adequate to explain the blocking by BTP: that the blocker interacts only with the open state of the channel, or, conversely, that the channel has the opportunity to close only when it is not blocked. We have not subjected the other organic blockers to these quantitative tests, but we have observed this kind of behavior qualitatively. We think that the behavior of BTP is typical of the other blockers studied here.

It is important to realize that the above effects of BTP on the channel kinetics do not imply that the blocker interacts directly with the channel's gating process. According to scheme 3, the gating and the blocking of the channel are entirely independent processes. The "coupling" between the two

processes arises only because of our measurement's inability to distinguish between the blocked and open states.

Dependence of Block on pH

Since BTP can bear either one or two charges, depending on the degree of dissociation of its two secondary amino groups (pKa = 9.0, 6.8), it was of
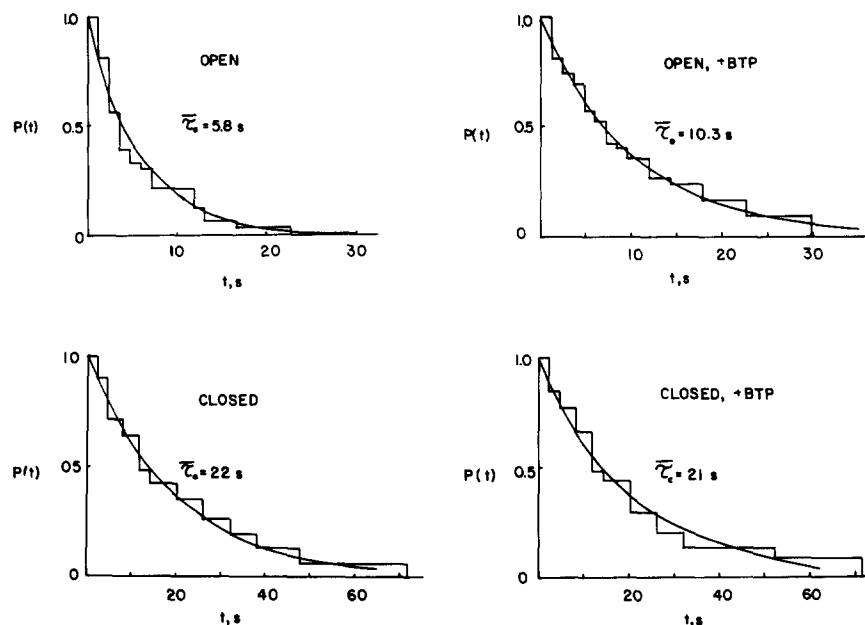

FIGURE 6.   Effect of BTP on single-channel kinetics. A membrane containing one fluctuating channel was obtained under the conditions of Fig. 5. After records involving about 80 transitions were obtained, 4.5 mM BTP was added to the *trans* chamber, and further records of about the same length were taken. Time histograms (Poisson plots) were then constructed by measuring the statistical distributions of dwell times in the open or closed states (Coronado et al., 1980), and these are plotted as cumulative distribution functions; i.e., $P(t)$ represents the probability of observing dwell time longer than time t. Mean open or closed times were measured (indicated on plots), $\bar{\tau}_i$, and these mean values were used to draw solid curves according to a Poisson law:

$$P(t) = \exp(-t/\bar{\tau}_i),\tag{6}$$

where $\bar{\tau}_i$ is the appropriate mean time above.

interest to determine the effect of pH upon the blocking reaction. Fig. 8 shows $\langle\gamma\rangle$ vs. V curves in the presence of 3 mM BTP at either pH 5.9 or pH 8.1. At the higher pH, the blocker is almost always singly charged; here, the effective valence is 0.57, close to the values seen for most monovalent blockers (Table II). At the lower pH, the molecule is now almost always doubly charged, and

the block becomes nearly twice as steeply voltage dependent, with effective valence of 1.1. The zero-voltage dissociation constant is also changed by variation in pH; the doubly charged species actually binds a bit more weakly ($K(0)$ = 20 mM at pH 5.9) than the singly charged species ($K(0)$ = 14 mM at pH 8.0). We cannot draw any conclusions from the pH dependence of the $K(0)$ because the channel itself may change its charge with pH (Labarca et al., 1980). The pH dependence of the effective valence, however, argues that the blocker can enter the channel in both of its charged forms.
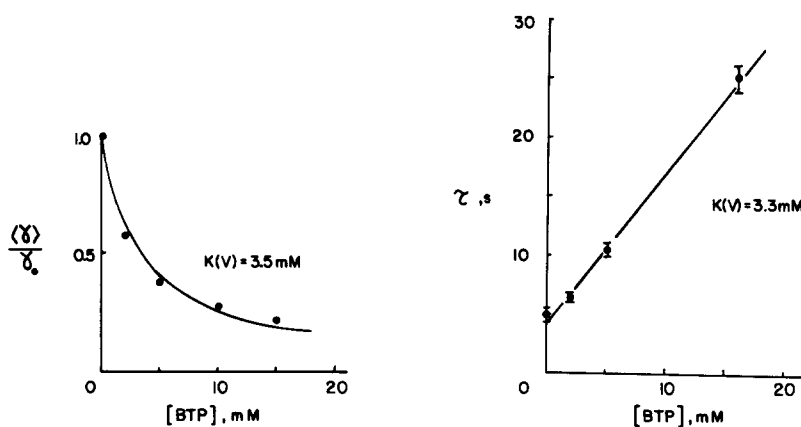

FIGURE 7. Concomitant effects of BTP on channel conductance and open time. Channel conductance and mean open times were measured as in Fig. 6, in the presence of various concentrations of BTP added to the *trans* side of the membrane. In the left panel, the relative conductance is plotted (means ± SEM of 4–10 measurements), and data are fit to Eq. 1, with blocking constant $K(V)$, measured at voltage V, shown. In the right panel, mean open times, $\bar{\tau}$, are plotted and a straight line was drawn by eye, according to Eq. 4; errors on points represent the extreme range determined by rocking the line on a linearized Poisson plot by eye. The blocking constant determined in this way according to Eq. 4 is also shown on the plot. No change in mean closed time was seen as BTP concentration was varied. Furthermore, the product $\bar{\tau}$ $\langle\gamma\rangle$ was measured for each point and was found to vary <10% (635 ± 60 pF). Voltage in this experiment was −30 mV.

DISCUSSION

Conduction by Small Organic Cations

In this study, we have made direct measurements of single-channel conductance of organic cations with various molecular structures. We find a rather sharp cutoff in conductance for molecules above 20 Å^2 in cross-sectional area, which suggests that the major selectivity region of this channel involves a constriction on the order of 20 Å^2. This estimate is smaller than the postulated dimension of the constriction of the nonselective acetylcholine-activated end-plate channel (35 Å^2; Horn and Stevens, 1980; Dwyer et al., 1980), and larger

than those postulated for the Ranvier node Na^+ (15 $\mathring{A}^2$; Hille, 1971a) and K^+ (9 $\mathring{A}^2$; Hille, 1975) channels. The size estimated here is in excellent agreement with that given by McKinley and Meissner (1978), 24 $\mathring{A}^2$, on the basis of ion flux experiments in SR vesicles; this is further evidence that the permeability pathway studied by McKinley and Meissner is the same as the SR K^+ channel studied in this laboratory.

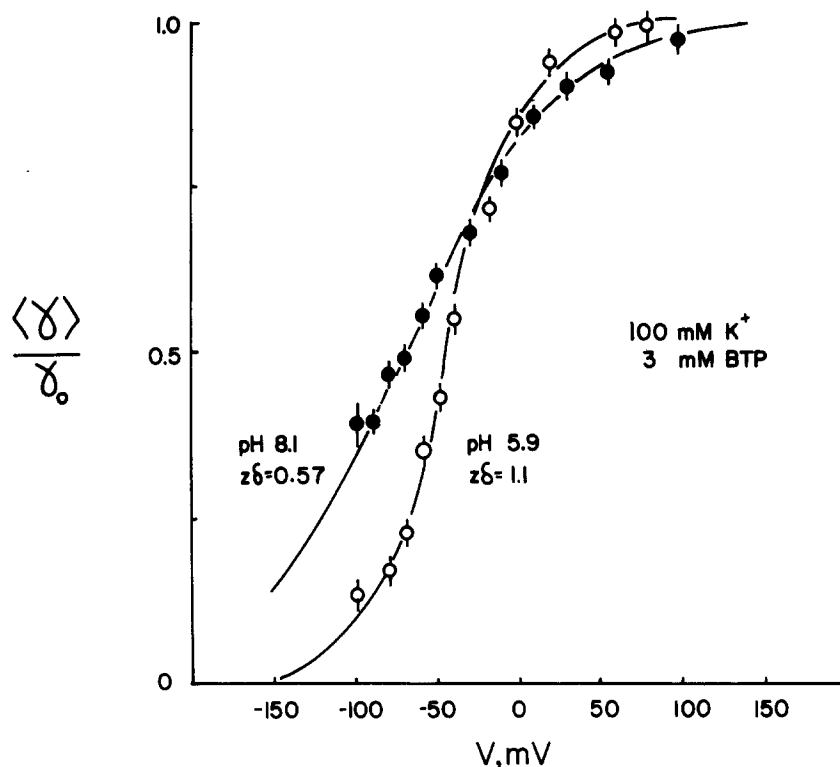There are several exceptions to this purely steric picture of ion exclusion.

FIGURE 8. pH-dependence of block by BTP. Blocking parameters of BTP were measured in PE membranes, with 3 mM BTP, as in Fig. 3, at either pH 5.9 (open points) or pH 8.1 (closed points), adjusted with Hepes or KOH. Each point represents the mean ± SEM of 4–10 determinations. Unblocked conductance is itself pH dependent (Labarca, 1980), and was 145 pS at pH 8.1 and 90 pS at pH 5.9. Solid curves were drawn according to Eq. 1, with $z\delta = 1.1$ and $K(0) = 20$ mM at pH 5.9, and $z\delta = 0.57$ and $K(0) = 14$ mM at pH 8.1.

First, there are a number of cations, such as TEA, with cross sections larger than 20 $\mathring{A}^2$ that do display discernable though small conductance. This is not an observation fatal to the model, however, because we might expect both the blocker molecule and the channel structure to possess some degree of flexibility.

Another exception is that of the isosteric series: ammonium (NH_3-H), hydrazinium (NH_3-NH_2), methylammonium (NH_3-CH_3). Viewed in silhou-

ette, these three molecules show identical cross sections, but we find that the channel conductances vary over a 2.7-fold range (156 pS for ammonium, and 58 pS for methylammonium). Another such case is the anomalously high conductances shown by the large molecules ethanolammonium (50 pS), formamidinium (36 pS), and methylhydrazinium (40 pS), all of which have silhouette cross sections close to 18 Å². A full explanation of these anomalies will require a complete description of the three parameters of ionic selectivity: permeability, binding affinity, and maximum conductance (Coronado et al., 1980). Because this detailed information is only now under initial study, we are unwilling to speculate upon the reasons for these anomalies. Nevertheless, we would like to draw attention to a molecular factor that seems to determine organic cation selectivity here and has also been seen in the Na^+ channel of Ranvier node (Hille, 1971a): the selection against methylated cations. In Table III we show several cases in which methylation reduces the channel

TABLE III
EFFECT OF METHYLATION ON CHANNEL
CONDUCTANCE

Ion	Cross section	γ
	$Å^2$	pS
Ammonium	13	156
Methylammonium	13	58
Methylhydrazinium	19	40
N,N-dimethylhydrazinium	21	12
Guanidinium	18	12
Methylguanidinium	18	4
Ethanolammonium	18	50
2-Methylaminoethanol	19	6

conductance three- to sixfold, with little or no change in cross-sectional area of the blocker.

Since methyl groups cannot participate in hydrogen bonding, Hille (1971a, 1975) argued that methylated derivatives are "seen" by the pore as larger than nonmethylated derivatives of nominally equivalent size. Cations with hydrogen-bonding groups can overlap their van der Waals radii with H acceptors in the channel, such as carbonyl oxygens. This explanation might also hold for the three cases presented in Table III, especially since the size of the cations is close to the limit of the maximum size able to cross the pore. Guanidinium, ethanolammonium, and methylhydrazinium would be forced to interact strongly with the walls of the pore, so as to make hydrogen bonding an important factor stabilizing a sterically unfavorable transition state in the passage of the ion. (It may seem that the exceptionally low conductance for hydroxylammonium [9 pS] argues against the above reasoning, but since at the pH of these experiments hydroxylamine is only 5–10% in charged form, this compound cannot be validly compared to the others.)

An additional effect of methylation can be seen by examining the results of blocking by quaternary ammonium derivatives. The affinity of block rises with increasing number of methylene groups, whether these are added to the "head" of symmetrical quaternary ammonium ions or to the "tail" of alkyl-trimethylammonium ions (Table II); this same effect has been observed in the axon K^+ channel (Armstrong, 1975a and b; Swenson, 1981; French and Shoukimas, 1981). If an increase in affinity of binding of the smaller, conducting cations also occurs upon methylation, this would also help to explain the lowered conductance of methylated ammonium derivatives with respect to their nonmethylated isosteric analogs.

Blocking by Large Organic Cations

For this discussion, we will assume that the effect of the blocking organic cations on the K^+ conductance arises by virtue of the entry of these ions into the normal diffusion pathway of the channel. As a result of this entry, the channel is unable to pass K^+ until the blocker leaves the channel. We do not consider any alternative modes of inhibition by blockers here. The evidence that supports this idea is: (a) the block by organic cations is competitive with K^+; (b) several "typical" blockers are trivial derivatives of ammonium ion, which itself conducts through this channel similarly to K^+; and (c) several of the blocking ions (TMA, TEA) also show small but recognizable conductance through the channel.

The major observation regarding organic cation blocking is that the position of the block is insensitive to the molecular structure of the blocker. This is a surprising result. We had originally intended to map the internal structure of the channel by varying the size of the blocker and observing a varying electrical distance of the block. Such a variation of electrical distance was not observed; even a molecule as large as glucosamine or tetrabutylammonium can still reach into the channel as far as, say, methylguanidinium. We must therefore argue that up to ~65% of the voltage drop occurs in a fairly wide region (at least 6 Å in diameter), and that this region ends suddenly at a constriction at which most of the organic blockers act. It seems natural to identify this constriction with the 20 $Å^2$ "selectivity region" postulated on the basis of the small organic cation conduction results.

These results have confirmed several aspects of the conduction model already suggested for the SR K^+ channel (Coronado et al., 1980). We previously postulated that one of the conduction sites for K^+ ion is located at 38% of the voltage drop measured from the cis side of the channel; this was based on the voltage dependence of the block by Cs^+ ion (Coronado and Miller, 1979), which exerts its blocking effect only from the cis side of the membrane. The position of the Cs^+ blocking site (38% from the cis side) corresponds well to the position of the organic cation blockers (63% from the trans side, from Table I). This also agrees well with the trans blocking by hexamethonium and decamethonium (Coronado and Miller, 1980; Miller, 1982), which independently supports the existence of a blocking site 60–65% of the potential drop from the trans side. We do not understand why Cs^+ exerts its blocking effect primarily from the cis side rather than from the trans.

The single-ion postulate previously offered for the channel (Coronado et al., 1980) is also in accord with the blocking data here. The block by organic cations (as with Cs^+) is purely voltage dependent; there is no indication of current dependence to the block (i.e., no discontinuity in the blocking effectiveness as the direction of K^+ current reverses), as would be expected for a knock-on mechanism (Armstrong, 1975b) in a multi-ion pore. Furthermore, we have never observed an electrical distance of block greater than unity, nor have we ever found this parameter to depend upon the concentration of either the blocker or of K^+, as might be expected for a multi-ion pore (Hille and Schwartz, 1978; Adelman and French, 1978).

Finally, the coupling of the channel's gating to the blocking reaction, as illustrated by the parallel effects of BTP upon time-averaged channel conductance and mean open time, argues that the simple "closed-open-blocked" scheme is an adequate description of the effects of these organic blockers on the channel. There is no need for additional states, such as "closed and blocked" states, as have been postulated for Na^+ channels (Lo and Schrager, 1981) and acetylcholine-gated channels (Eldefrawi et al., 1980).

The general picture of a channel structure containing a wide, nonselective "mouth" in series with a narrow constriction at which ionic selectivity occurs is by now quite familiar. Studies using blocking ions have led to this sort of hypothesis for the squid axon K^+ and Na^+ channels (Armstrong, 1975b; Swenson, 1981; French and Shoukimas, 1981; Rojas and Rudy, 1976), for the acetylcholine-activated endplate channel (Horn and Stevens, 1980), and for the *Torpedo* electroplax Cl^- channel (White and Miller, 1981). We can now add another channel to the list of those conforming to this picture, the K^+ channel of SR. These are the only integral membrane channels that have been studied in detail by this method, and all five of them appear to present the conducting ions with a funnel-like diffusion pathway. The question then arises whether this feature will turn out to be a general structural trait of channels from higher organisms.

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